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Comm. Resp. to Examiner Inquiry dated November 22, 2004

EXHIBIT E

A copy of pages 83-84 of the instant application providing the amino acid sequence of a Pv-FRIL (SEQ ID NO:6).

reported above. The full lengthed product was cloned in the EcoRI site of the cloning vector pCR2.1 (Fig. 24A) and sequenced as noted above. This plasmid is referred to as pCR2.1-Pv-FRIL.

The nucleic acid sequence of the Pv-FRIL cDNA is as follows:

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   1  GCTCAGTCAT TATCTTTTAA CTTTACCAAG TTTGATCTTG ACCAAAAAGA
  51  TCTTATCTTC CAAGGTGATG CCACTTCTAC AAACAATGTC TTACAACTCA
 101  CTAAGTTAGA CAGTGGAGGA AACCCTGTGG GTGCTAGTGT GGGAAGAGTG
 151  TTATTCTCTG CACCATTTCA TCTTTGGGAA AACTCTATGG CAGTGTCAAG
 201  CTTTGAAACT AATCTCACCA TTCAAATCTC AACACCTCAC CCTTATTATG
 251  CAGCTGATGG CTTTGCCTTC TTCCTTGCAC CACATGACAC TGTCATCCCT
 301  CCAAATTCTT GGGGCAAATT CCTTGGACTC TACTCAAACG TTTTCAGAAA
 351  CTCCCCCACC TCTGAAAACC AAAGCTTTGG TGATGTCAAT ACTGACTCAA
 401  GAGTTGTTGC TGTCGAATTT GACACCTTCC CTAATGCCAA TATTGATCCA
 451  AATTACAGAC ACATTGGAAT CGATGTGAAC TCTATTAAGT CCAAGGAAAC
 501  TGCTAGGTGG GAGTGGCAAA ATGGGAAAAC GGCCACTGCA CGCATCAGCT
 551  ATAACTCTGC CTCTAAAAAA TCAACTGTTA CTACGTTTTA TCCTGGGATG
 601  GAAGTTGTGG CTCTCTCCCA TGATGTTGAC TTACATGCAG AGCTTCCTGA
 651  ATGGGTTAGA GTAGGGTTAT CTGCTTCAAC TGGAGAGGAG AAACAAAAAA
 701  ATACCATTAT CTCATGGTCT TTCACTTCAA GCTTGAAGAA CAACGAGGTG
 751  AAGGAGCCGA AAGAAGACAT GTATATTGCA AACGTTGTGC GATCATATAC
 801  ATGGATCAAT GACGTTCTAT CTTATATAAG CAATAAATAA ATGTATGATG
 851  CACTCAATAA TAATCACAAG TACGTACGGT GTAGTACTTG TATGTTGTTT
 901  ATGAAAAAAA AAAA                                (SEQ ID NO: 5)
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The amino acid sequence of Pv-FRIL is as follows:

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AQSLSFNFTKFDLDQKDLIFQGDATSTNNVLQLTKLDSGGNPVGASVGRVLFSAPFHLWENSMAV
SSFETNLTIQISTPHPYYAADGFAFFLAPHDTVIPPNSWGKFLGLYSNVFRNSPTSENQSFGDVN
TDSRVVAVEFDTFPNANIDPNYRHIGIDVNSIKSKETARWEWQNGKTATARISYNSASKKSTVTT
FYPGMEVVALSHDVDLHAELPEWVRVGLSASTGEEKQKNTIISWSFTSSLKNNEVKEPKEDMYIA
NVVRSYTWINDVLSYISNK*MYDALNNNHKYVRCSTCMLFMKKK  (SEQ ID NO: 6)
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The amino acid sequence of Pv-FRIL was compared to the amino acid sequences of Dl-FRIL and of the PHA-E lectin. This comparison is shown on Fig. 24B.

Pv-FRIL-Encoding Plant Expression Vectors and *Nicotiana tabacum* Transformation

Recombinant PCR was used to introduce a signal peptide for entry of Pv-FRIL into the endoplasmic reticulum at the 5' end of the Pv-FRIL cDNA clone. Following the procedure of Higuchi (*supra*) the signal peptide and the full length cDNA clone were amplified in two separate primary PCR reactions. The signal peptide was obtained from the amplification of the binary vector pTA4, harboring the complete sequence of the bean α-amylase inhibitor gene (Hoffman et al., L.M., Y. Ma and R.F. Barker, *Nucleic Acid Res.* 10: 7819-7828, 1982; Moreno and Chrispeels, *Proc. Natl. Acad. Sci. USA* 86: 7885-7889, 1989).

The primers used for the two primary reactions are the following:

Amplification of the Signal Peptide

Sigforw BglII: AGA TCT ATG GCT TCC TCC AAC

Sigrew: AAA GAT AAT GAC TGA GCG GCT GAG TTT GCG TG

Amplification of the mannose lectin cDNA:

SpMIforw: CAC GCA AAC TCA GCC GCT CAG TCA TTA TCT TT

APXhoI: CTC GAG GAC CAC GCG TAT CGA TGT CGA

The primers used for the secondary reactions, Sigforw and AP, were designed to generate BglII sites at the 5' and XhoI at the 3' ends. Both, primary and secondary PCR reactions were performed as discussed above. The recombinant product SpPv-FRIL was incubated for 10 min. at 72°C with 0.5 units of Ampli-Taq polymerase (Perkin Elmer) and cloned in the cloning vector pCR2.1 (Fig. 25). The nucleotide sequence of the PCR product was determined as described above to verify the correct attachment of the signal peptide.